O. B. Trifonova et al /J. Pharm. Sci. & Res. Vol. 10(12), 2018, 3164-3166

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

The Effect of Hawthorn Berries (Crataegus spp.) Granulation Process on Biologically Active Compounds Content

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Abstract

Expansion of herbal medicines product range can be achieved in several ways, including development of novel dosage forms. Currently, two most popular dosage forms for herbal drug products are herbal infusions and decoctions; their popularity is justified by safety and effectiveness, possibility of long-term use, low price, and high availability.

Herbal infusions and decoctions are usually prepared from cut or powdered herbal material by aqueous extraction. However, cut or powdered materials have inadequate technological properties, e.g. low flowability, which can affect their dosing, especially on modern high-performance manufacturing lines. This leads to a necessity of pre-treatment that is required for most powdered herbal drugs released in tea filter bags (herbal teas). These aspects have led to the development of cut-pressed granules – a unique novel dosage form.

Manufacturing of granules from herbal material is feasible only if the quality of the material does not decrease during granulation process. Previously, it was established that morphological and anatomical characteristics of several types of herbal raw material, including hawthorn (*Crataegus spp.)* berries, are not affected by granulation process.

The aim of our research was to investigate the effect of manufacturing process on the qualitative and quantitative content of biologically active substances in hawthorn berries powder (four commercial batches) and cut-pressed granules (four pilot batches). Spectrophotometric analysis of total flavonoid content showed that it lies within 0.04-0.09% range and is not affected by granulation process. It was also found that the chromatographic characteristics of alcohol extracts (obtained by modified Eur. Ph. method) from hawthorn berries and hawthorn cut-pressed granules are similar. Overall, the results of the study confirm that the granulation process does not alter content of biologically active compounds in both hawthorn berry powder and cut-pressed granules.

Keywords: cut-pressed granules, differential spectrophotometry, hawthorn berries, flavonoids, thin-layer chromatography.

INTRODUCTION

An evergrowing demand for novel medicinal products with complex therapeutic activity stipulates the need for expanding the range of herbal medicines. This is commonly achieved by development of either new active pharmaceutical ingredients (AFIs) of plant origin or innovative dosage forms. Currently, the latter remains a key area of focus for pharmaceutical industry due to several reasons, including possibility to improve technological process efficiency, decrease production costs, lower in-process material loss, etc.

Highest yield of biologically active substances during aqueous extraction is achieved by a combination of factors affecting extraction process, the most important of them being the particle size: it is impossible to extract required amount of substances from large particles, whereas extraction from particles that are too fine can contaminate the extract with inert substances and plant tissue fragments. Besides, cut or powdered materials have inadequate technological properties, e.g. low flowability, which can affect its dosing, especially on modern high-performance manufacturing lines. Therefore, a special pre-treatment is required for most powdered herbal drugs released in tea filter bags (herbal teas) [1-3]. These aspects have led to the development a unique novel dosage form - cut-pressed granules (http://www.femb.ru/feml) [4].

Manufacturing of cut-pressed granules from herbal raw material is feasible only if the qualitative and quantitative content of biologically active substances is not altered by granulation process [5]. In each specific case, experimental studies should confirm that the quality of granules from herbal raw material is not inferior to that of the powdered material.

Previously, we have shown that the granulation process does not affect morphological and anatomical characteristics of hawthorn berries [6] and certain other herbal raw materials [7]. Therefore, the aim of our study was to investigate the effect of cut-pressed granules manufacturing process on qualitative and quantitative content of biologically active substances in powdered hawthorn berries and cut-pressed granules, derived from the powder.

MATERIALS AND METHODS

The objects of the study were commercial batches of powdered hawthorn berries (quality corresponds to the "Hawthorn berries (*Fructus Crataegi*)" monograph requirements) [8], and pilot batches of cut-pressed granules derived from the powder. All reagents and solvents used in the study were of analytical grade and were used as received without further purification. Hyperoside reference standard (CAS 482-36-0) was obtained from PhytoLab (Germany).

Weighing of samples was performed using GH-252 (AND, Japan) analytical balance.

Powdered herbal raw material, passing through a 2 mm sieve, was used for manufacturing of cut-pressed granules. The powder was moistened for 3-4 minutes using saturated steam (vapor pressure – 3.5-5.5 kgf/cm^2) under constant stirring for even moist distribution. After that the material was transferred to a compression machine in which the moistened mass was pushed through a 5-7 mm sieve. The material was extruded from the machine in form of 10-30 mm cylinders, which were transferred to the dryer. After drying, the material was force-cooled with air and was transferred to a roll grinder in which it was crushed to granules passing through a 2 mm sieve.

Comparative qualitative analysis of phenolic compounds in hawthorn berry powder and cut-pressed granules was performed by thin-layer chromatography (TLC), using "TLC Silica gel 60 F254" aluminum TLC plates (Merck, Germany). Extraction of biologically active substances from the material was performed using modified European Pharmacopoeia procedure [9]: about 1.0 g of the powdered berries, passing through a 0.5 mm sieve, was placed in a 100 ml ground glass flask and 10 ml of 96% ethyl alcohol were added. The flask was heated under backflow condenser on a water bath at 65^{o}C for 5 minutes. After cooling to room temperature, the content of the flask was filtered through a paper filter into a graduated flask, obtaining 10 ml of the filtrate (*Test Solution*).

Bands of 30 µl (0.03 ml) of *Test Solution* and 2 µl (0.002 ml) of hyperoside reference standard (*Standard Solution*) were applied to the start line of the chromatographic plate.

A mixture of ethyl acetate, acetone, toluene, anhydrous formic acid, and water (20:10:10:5:5) was used as a mobile phase. Ascending chromatography was performed in TLC chamber (Camag, Switzerland).

Two detection solutions were consequently used in the experiment: 10 g/L solution of diphenylboric acid aminoethyl ester in 96% ethyl alcohol (*Detection Solution 1*) and 50 g/L polyethylene glycol 400 solution in 96% ethyl alcohol (*Detection Solution 2*).

A "Reprostat 3" TLC imaging system (Camag, Switzerland) was used to obtain photographs of the chromatogram. The photographs were processed using Adobe Photoshop 7.0 (Adobe, USA).

The effect of granulation process on the total flavonoid content (expressed as hyperoside) was assessed using differential spectrophotometry [10]. About 0.5 g (exact weight) of the powdered material, passing through a 1 mm sieve, were placed in a 250 ml ground glass flask, 50 ml of 96% ethyl alcohol were added, and the flask was heated on a boiling water bath for 60 minutes under backflow condenser. After cooling to room temperature, the liquid content of the flask was carefully filtered through a paper filter into 100 ml volumetric flask, and 30 ml of 96% ethyl alcohol were added to the herbal material in the 250 ml flask. The flask was again subjected to the heating on the boiling water bath for 60 minutes under backflow condenser. After cooling, liquid content was filtered through the same filter into the same volumetric flask. The cycle was repeated one more time, using 20 ml portion of 96% ethyl alcohol. The content of the volumetric flask was then brought to volume with 96% ethyl alcohol (*Test Solution A*).

Ten (10) ml of *Test Solution A* were placed into 25 ml volumetric flask and 6 ml of 2% alcoholic solution of aluminum chloride were added to its content. The solution was brought to volume with 96% ethyl alcohol and mixed (*Test Solution* B). Absorbance of the *Test Solution B* was measured using Cary 50 UV-Vis spectrophotometer (Varian, USA) at 410 nm in 10 mm cuvette. The solution containing 10 ml of *Test Solution A* and 0.1 ml of concentrated acetic acid, placed into 25 ml volumetric flask and brought to volume with 96% ethyl alcohol, was used as the *Reference solution.*

Total flavonoid content (TFC), expressed as per cent (X) of hyperoside equivalent per g of dry material, was calculated using the following formula:

$$X = \frac{A \cdot 100 \cdot 25 \cdot 100}{380 \cdot a \cdot 10 \cdot (100\text{-}W)} = \frac{A \cdot 2500}{a \cdot 38 \cdot (100\text{-}W)}$$

where:

A – absorbance of *Test Solution B*;

380 – specific absorbance of hyperoside-AlCl3 complex at 410 nm;

a – mass of analyzed sample, g;

W – herbal raw material moisture content, %.

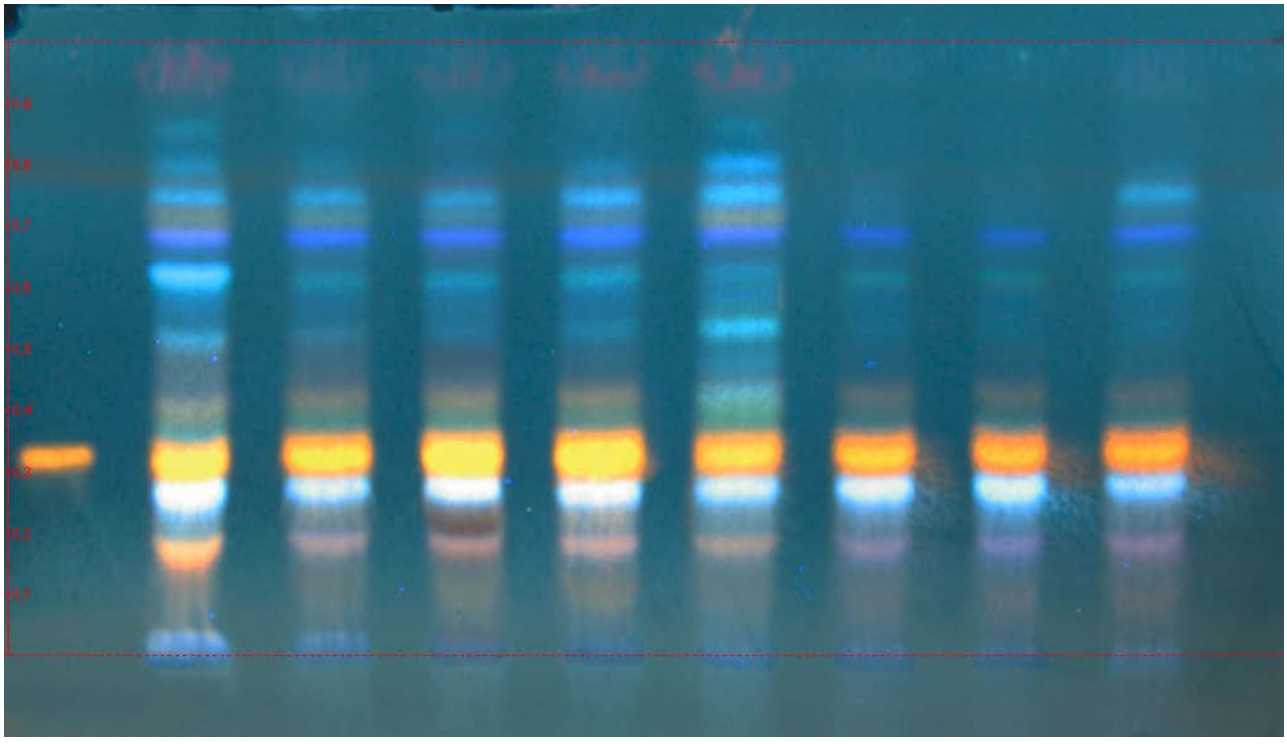

Figure 1. Photo of chromatogram of phenolic compounds obtained using hawthorn berries powder and hawthorn cut-pressed granules, after spraying with 1% alcoholic solution of diphenylboric acid aminoethyl ester and 5% alcoholic solution of polyethylene glycol 400 (365 nm UV light):
1 – hyperoside reference standard (0.025% alcoholic solution), 2 µl
2 – aqueous extract from hawthorn cut-pressed granules, Batch No. 40216, 30 µl
3 – aqueous extract from hawthorn cut-pressed granules, Batch No. 121016, 30 µl
4 – aqueous extract from hawthorn cut-pressed granules, Batch No. 20217, 30 µl
5 – aqueous extract from hawthorn cut-pressed granules, Batch No. 121217, 30 µl
6 – aqueous extract from hawthorn berries powder, Batch No. 40216, 30 µl
7 – aqueous extract from hawthorn berries powder, Batch No. 121016, 30 µl
8 – aqueous extract from hawthorn berries powder, Batch No. 20217, 30 µl
9 – aqueous extract from hawthorn berries powder, Batch No. 121217, 30 µl

Table 1. Results of TFC determination in hawthorn berries powder and hawthorn cut-pressed granules (mean of 3 measurements)

Hawthorn berries		Hawthorn cut-pressed granules	
Batch No.	**TFC expressed as hyperoside, %**	**Sample No.**	**TFC expressed as hyperoside, %**
40214	0.04	Cut-pressed granules from batch 40214	0.05
10215	0.07	Cut-pressed granules from batch 10215	0.06
20315	0.06	Cut-pressed granules from batch 20315	0.06
50915	0.05	Cut-pressed granules from batch 50915	0.05
20116	0.09	Cut-pressed granules from batch 20116	0.09
30216	0.04	Cut-pressed granules from batch 30216	0.05
40216	0.04	Cut-pressed granules from batch 40216	0.05
50216	0.08	Cut-pressed granules from batch 50216	0.09
60316	0.05	Cut-pressed granules from batch 60316	0.05
121016	0.04	Cut-pressed granules from batch 121016	0.05
20217	0.04	Cut-pressed granules from batch 20217	0.05
121217	0.05	Cut-pressed granules from batch 121217	0.06

RESULTS AND DISCUSSION

After solvent front has passed about 80-90% of the TLC plate length from the start line, the plate was removed from the chamber and air-dried until full evaporation of solvent residues. The chromatogram was sprayed with *Detection Solution 1*, dried, then sprayed with *Detection Solution 2* and immediately placed in the temperature chamber at 100-105 ºC for 1-3 minutes. The chromatogram is inspected under UV light (365 nm wavelength).

The chromatogram of *Reference Solution* in the lower half shows zone of yellow, greenish-yellow, yellow-green, or yellow-orange color (hyperoside).

The chromatogram of *Test solution* shows the following zones: a yellow, greenish-yellow, yellow-green, or yellow-orange zone at the level of hyperoside standard zone; a light-blue or blue zone above the zone due to hyperoside; zones of pinkish-violet, yellow, yellowish-green or yellowish-orange, and light-blue zones below the zone due to hyperoside.

Chromatographic profiles of powdered hawthorn berries and pilot samples of hawthorn cut-pressed granules were similar (Figure 1).

The data obtained during spectrophotometric analysis of commercial batches of hawthorn berry powder and pilot samples of cut-pressed granules have shown that TFC is between 0.04 and 0.09%, and the granulation process does not alter biologically active substances content (Table 1).

CONCLUSION

Thus, by means of spectrophotometric analysis it was demonstrated that TFC of both hawthorn berry powder and hawthorn cut-pressed granules lies within 0.04-0.09% range and is not affected by granulation process. Results of chromatographic analysis allow us to suggest that the qualitative content of the biologically active substances remains the same in both objects of the study, since the chromatographic characteristics of studied extracts are similar. It can be concluded, that the granulation process does not alter content of biologically active compounds in both hawthorn berry powder and cut-pressed granules.

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